BIOSPECTRA

100 Majestic Way, Bangor, PA 18013 / www.biospectra.us

SODIUM HYDROXIDE 5N
TESTING METHODS

TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide Laboratory personnel with a procedure for analyzing Sodium Hydroxide 5 N In-Process, Stability, and Finished Good samples.

2. SCOPE:

2.1. Applies to the analysis of Sodium Hydroxide 5N In-Process, Stability, and Finished Goods in the Laboratory. Methods include testing for all grades of Sodium Hydroxide 5N sold by BioSpectra; only the specific tests required for the requested grade must be tested.

3. RESPONSIBILITIES:

3.1. The Laboratory Manager, or qualified, designee is responsible for training, maintenance and implementation of this procedure.

3.2. Laboratory personnel are responsible for compliance with the terms of this procedure. This includes notifying Laboratory Management if any analyses fail to meet their respective specifications.

4. SAFETY:

4.1. Causes SEVERE skin burns and eye damage. Standard laboratory safety regulations apply. Before working with any chemical, read and understand the Safety Data Sheet (SDS).

5. REFERENCES:

5.1. BSI-MEM-0130, Endosafe NexGen PTS Endotoxin Reader: Qualified Products
5.2. BSI-SOP-0019, Result Reporting
5.3. BSI-SOP-0098, Balance SOP
5.4. BSI-SOP-0126, Laboratory Notebooks
5.5. BSI-SOP-0135, Laboratory Chemicals
5.6. BSI-SOP-0140, Standardization of Titrants
5.7. BSI-SOP-0242, Bangor Portable Turbidimeter and Calibration SOP
5.8. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
5.9. BSI-SOP-0303, NexION 350X ICP-MS SOP
5.10. BSI-SOP-0350, Anton Paar DMA 35 Portable Density Meter Operation and Calibration
5.11. BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP
5.12. *ACS Reagent Chemicals*, current edition
5.13. *USP-NF* current edition

6. EQUIPMENT:

6.1. Analytical Balance
6.2. Hach Portable Turbidimeter Model 2100 Q, or equivalent
6.3. Calibrated Oven
6.4. Anton Paar DMA 35 Portable Density Meter
6.5. Endosafe PTS Endotoxin Reader, or equivalent
6.6. NexION 350X ICP-MS

7. REAGENTS:

7.1. **0.02N Hydrochloric Acid (HCl):** Slowly add 20 mL of 0.1N Hydrochloric Acid to 80 mL of purified water to make a total volume of 100 mL.
7.2. **1N Acetic Acid:** Dilute 57mL of glacial acetic acid to 1L with purified water.
7.3. **10% Ammonium Hydroxide:** Dilute 35mL of 29% ammonium hydroxide to 100mL with purified water.
7.4. **Ammonium Peroxydisulfate Crystals:** purchased commercially.
7.5. **Ammonium Thiocyanate:** purchased commercially.
7.6. **30% Ammonium Thiocyanate:** Dissolve 30g of ammonium thiocyanate in water and dilute with water to 100mL.
7.7. **Endosafe PTS Cartridge 1-0.01 EU/mL:** purchased commercially.
7.8. **Ferrous Ammonium Sulfate Hexahydrate:** purchased commercially.
7.9. **Glacial Acetic Acid, concentrated:** purchased commercially.
7.10. **Glycerin:** purchased commercially.
7.11. **Glycerin Base:** To 200g of glycerin, add water to a total weight of 235g. Add 140mL of 1N NaOH, 50mL of purified water and mix.
7.12. **Hydrochloric Acid, concentrated:** purchased commercially.
7.13. **Iron Standard (0.01mg of Fe in 1mL):** Dissolve 0.702g of ferrous ammonium sulfate hexahydrate in 10mL of 10% sulfuric acid and dilute with water to 100mL. To 10mL of this solution, add 10mL of 10% sulfuric acid and dilute with water to 1L.
7.14. **LAL Reagent Water:** purchased commercially.
7.15. **Lead Nitrate:** purchased commercially.
7.16. **Lead Stock Solution (0.1mg of Pb in 1mL):** Dissolve 0.160g of lead nitrate in 100mL of dilute nitric acid (1:99) and dilute with purified water to 1L. The solution should be prepared and stored in containers free from lead.
7.17. **Litmus Paper:** purchased commercially
7.18. **Nitric Acid (HNO_3):** purchased commercially
7.19. **Nitric Acid, dilute (1:99):** Dilute 1mL of 69% nitric acid in 99mL of purified water.
7.20. **Phenolphthalein:** purchased commercially.
7.21. **Phenolphthalein Indicator:** Dissolve 1.0 g of phenolphthalein in 100 mL of reagent grade alcohol.
7.22. **pH 9-10 Buffer (0.25M Tris Base):** purchased commercially.
7.23. **Potassium Carbonate (15%):** Weigh 15.000g of Potassium Carbonate and transfer to a 100mL volumetric flask. Dissolve and dilute to volume with purified water.
7.24. **Potassium Hydrogen Phthalate (KHP):** Crush and dry a suitable amount of KHP at 120°C for 2 hours. Allow to cool to ambient temperature in a desiccator.
7.25. **Potassium Pyroantimonate TS:** purchased commercially.
7.26. **Purified Water: g**enerated in-house
7.27. **0.1N Silver Nitrate ($AgNO_3$) TS:** purchased commercially
7.28. **Thioacetamide:** purchased commercially.
7.29. **Thioacetamide TS:** Dissolve 4g of thioacetamide in purified water to make 100mL.

8. ANALYTICAL PROCEDURES:

8.1. IN-PROCESS TESTING:

8.1.1. **NORMALITY (CONFIRMATION 1 AND 2) REFER TO BATCH RECORD:**

8.1.2. **KHP (Potassium Hydrogen Phthalate) Preparation:**

8.1.2.1. Crush and dry a suitable amount of KHP at 120°C for 2 hours. Allow to cool to ambient temperature in a desiccator.

8.1.3. **Burette Preparation:**

8.1.3.1. Fill a 25-mL volumetric flask with sample. Quantitatively transfer the aliquot to a 250-mL volumetric flask with purified water. Rinse the 25-mL flask by filling the flask halfway with purified water, shaking it, then transferring the rinse to the 250-mL volumetric flask. Perform the rinse procedure in duplicate. Fill the 250-mL volumetric flask to volume with purified water. Mix well and cool to 25° ± 2°C. QS the sample solution to 250 mL after cooling is complete.

8.1.3.2. Prime the 50-mL burette by filling it with the diluted sample solution. Empty the burette and repeat.

8.1.3.3. Fill the burette to the required volume with the prepared sample solution.

8.1.4. **Sample Preparation:**

8.1.4.1. Weigh 4.0 – 4.2 g of the previously dried KHP into a 250-mL beaker.

8.1.4.2. Add 100 mL of purified water down the sides of the beaker to avoid the loss of KHP.

8.1.5. **Analysis Procedure:**

8.1.5.1. To the KHP solution, add 150 µL phenolphthalein indicator.

8.1.5.2. Titrate the KHP using the sample solution in the burette, to a pink endpoint.

8.1.5.3. Calculate the normality using the following equation:

$$N = \frac{KHP\ Weight\ (g) \times KHP\ Purity\ \times 10}{0.20423\ \times mL\ of\ NaOH\ Sample\ Solution}$$

Where:

$$KHP\ Purity = \frac{Assay\ percent\ of\ KHP}{100} \quad (from\ manufacturer's\ CoA)$$

$$0.20423 = \frac{Formula\ weight\ of\ KHP}{1000}$$

$$10 = Dilution\ Factor$$

8.2. **FINISHED GOOD TESTING:**

8.2.1. **APPEARANCE AND COLOR:**

8.2.1.1. Transfer 2 mL of sample into a 4 mL (10-mm) glass comparison tube.

8.2.1.2. Transfer 2 mL of purified water into a separate 4 mL (10-mm) glass comparison tube.

8.2.1.3. View the tubes vertically against a color comparison plate with suitable lighting. In order to pass, the test solution is complete, clear, and colorless when compared to purified water.

8.2.1.4. For Stability Testing: If the sample does not pass specification when compared to purified water, if can be compared to another sample determined to be passing (such as the Finished Goods lot retain) as a direct comparison to make the qualitative determination for Appearance and Color.

8.2.2. **CHLORIDE:**

8.2.2.1. Thoroughly rinse Nessler tubes using purified water prior to use.

8.2.2.2. **Sample Preparation:**

8.2.2.2.1. Weigh 2.0 g of sample and quantitatively transfer to a 50-mL Nessler Color Comparison Tube using purified water.

8.2.2.2.2. Dilute to ~20 mL with purified water.

8.2.2.2.3. Slowly, using extreme caution, acidify the sample with nitric acid to litmus.

8.2.2.2.4. Dilute to 40 mL with purified water.

8.2.2.3. **5 ppm Standard Preparation:**

8.2.2.3.1. Dilute 14.1 µL of 0.02N HCl to ~40 mL with purified water.

8.2.2.4. **Analysis:**

8.2.2.4.1. To both the sample and standard solutions, add 1 mL of concentrated nitric acid and 1 mL of 0.1N Silver Nitrate TS. Dilute the sample and standard to 50 mL with purified water.

8.2.2.4.2. Mix and allow solutions to sit for 5 minutes using a calibrated timer.

8.2.2.4.3. After 5 minutes, the turbidity in the sample solution does not exceed the turbidity produced by the standard when viewed against a dark background. Analyze turbidity utilizing the turbidity meter and record the sample NTU results.

8.2.3. **ENDOTOXIN:**

8.2.3.1. Pipette 0.200 mL of sample into a sterile vial and add 1.600 mL of LAL reagent water.

8.2.3.2. Check the pH of the solution with pH paper.

8.2.3.2.1. If the solution is basic, add HCl in small increments until the solution is acidic.

8.2.3.3. Once acidic, add sufficient pH 9-10 buffer solution until the pH is between 6-8.

8.2.3.4. Dilute to 10 mL with LAL reagent water.

8.2.3.5. Follow the Endosafe Nexgen PTS Endotoxin Reader SOP for sample analysis.

8.2.3.5.1. The dilution factor is 50.

8.2.4. **HEAVY METALS (Pb):**

Primary Method

8.2.4.1. Standard and Solution Preparation:

8.2.4.1.1. Lead Standard Solution (0.01mg of Pb in 1mL): Dilute 10mL of lead stock solution to 100mL with purified water. This must be prepared at the time of use.

8.2.4.1.2. Thioacetamide-glycerin base: Thoroughly mix 1mL of thioacetamide with 5mL of Glycerin base. Heat in a boiling bath for 20 seconds. Prepare immediately before use.

8.2.4.2. Procedure:

8.2.4.2.1. Note: Prepare in hood, and use caution for standard and sample preparation to avoid spattering of sample.

8.2.4.2.2. Sample Preparation: Weigh 30g of sample into a suitable beaker and carefully add 9mL of concentrated nitric acid.

8.2.4.2.3. Standard Preparation: Weigh 10g of sample and add 3mL of concentrated nitric acid. Add 2mL of 0.01mg Lead Standard Solution (0.01mg/mL Pb).

8.2.4.2.4. Place both the standard and sample on a hot plate and evaporate to dryness. Cool and dissolve each residue with 20 mL of purified water. Adjust the pH to between 3 and 4 utilizing a pH meter, with 1N acetic acid or 10% ammonium hydroxide.

8.2.4.2.5. Transfer the solutions to separate Nessler Color Comparison tubes. Add 1.2mL of freshly prepared thioacetamide-glycerin base to each of the solutions and mix. QA each tube to 50mL and mix.

8.2.4.2.6. Any brown color produced in the sample solution must not exceed that in the standard solution to be reported as ≤1 ppm.

Alternate Method

8.2.4.3. Refer to NexION 350X ICP-MS SOP.

8.2.5. **IDENTIFICATION (SODIUM):**

8.2.5.1. Pipette 1 mL of sample into a test tube containing 25 mL of purified water.

8.2.5.2. Add 2 mL of 15% Potassium Carbonate and heat to boiling.

8.2.5.3. Allow to cool in an ice batch and as necessary, rub the inside of the test tube with a glass rod to initiate precipitation.

8.2.5.4. Add 4 mL of Potassium Pyroantimonate TS and heat to boiling.

8.2.5.5. Allow to cool in an ice bath and as necessary, rub the inside of the test tube with a glass rod to initiate precipitation.

8.2.5.6. A dense precipitate must form in order to pass test.

8.2.6. **IRON (Fe):**

Primary Method

8.2.6.1. Procedure:

8.2.6.1.1. Sample Preparation: To 20g of sample, add 0.1mL of phenolphthalein indicator solution, neutralize with hydrochloride acid and dilute with water to 40mL.

8.2.6.1.2. 0.01mg Iron Standard Preparation: Pipette 1mL of Iron Standard (0.01mg of Fe in 1mL) and dilute with water to 40mL.

8.2.6.1.3. To the sample and standard solutions, add 30-50mg of ammonium peroxydisulfate crystals, 2mL of hydrochloric acid, and 3mL of 30% Ammonium Thiocyanate and mix.

8.2.6.1.4. Any red color in the sample must not exceed the 0.01mg standard solution to report as <0.5ppm.

Alternate Method

8.2.6.2. Refer to NexION 350X ICP-MS SOP.

8.2.7. **NORMALITY:**

8.2.7.1. **KHP (Potassium Hydrogen Phthalate) Preparation:**

8.2.7.1.1. Crush and dry a suitable amount of KHP at 120°C for 2 hours. Allow to cool to ambient temperature in a desiccator.

8.2.7.2. **Burette Preparation:**

8.2.7.2.1. Fill a 25-mL volumetric flask with sample. Quantitatively transfer the aliquot to a 250-mL volumetric flask with purified water. Rinse the 25-mL flask by filling the flask halfway with purified water, shaking it, then transferring the rinse to the 250-mL volumetric flask. Perform the rinse procedure in duplicate. Fill the 250-mL volumetric flask to volume with purified water. Mix well and cool to 25° ± 2°C. QS the sample solution to 250 mL after cooling is complete.

8.2.7.2.2. Prime the 50-mL burette by filling it with the diluted sample solution. Empty the burette and repeat.

8.2.7.2.3. Fill the burette to the required volume with the prepared sample solution.

8.2.7.3. **Sample Preparation:**

8.2.7.3.1. Weigh 4.0 – 4.2 g of the previously dried KHP into a 250-mL beaker.

8.2.7.3.2. Add 100 mL of purified water down the sides of the beaker to avoid the loss of KHP.

8.2.7.4. **Analysis Procedure:**

8.2.7.4.1. To the KHP solution, add 150 µL phenolphthalein indicator.

8.2.7.4.2. Titrate the KHP using the sample solution in the burette, to a pink endpoint.

8.2.7.4.3. Calculate the normality using the following equation:

$$N = \frac{KHP\ Weight\ (g) \times KHP\ Purity\ \times 10}{0.20423\ \times mL\ of\ NaOH\ Sample\ Solution}$$

Where:

$$KHP\ Purity = \frac{Assay\ percent\ of\ KHP}{100} \quad (from\ manufacturer's\ CoA)$$

$$0.20423 = \frac{Formula\ weight\ of\ KHP}{1000} \qquad 10 = Dilution\ Factor$$